

Appl. No. 10/004,115
Supplement Amdt. dated February 13, 2004

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

Claim 1 (previously presented): A polynucleotide sequence having:

- a) a polynucleotide sequence coding for an amino acid sequence represented by SEQ ID NO: 1;
- b) a polynucleotide sequence that hybridizes with a polynucleotide sequence coding for an amino acid sequence represented by SEQ ID NO: 1 to form a hybrid under high ion concentrations of 900mM sodium chloride and 90mM sodium citrate at 65°C and the hybrid formed is maintained as a hybrid after being kept at 65°C for 30 minutes under a low ion concentration of 15mM sodium chloride and 1.5mM sodium citrate, the amino acid sequence being an amino acid sequence of an enzyme capable of preferentially producing (S)-4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanotate;
- c) a an isolated polynucleotide sequence represented by SEQ ID NO: 2,
- d) a polynucleotide coding for an amino acid sequence of SEQ ID NO: 1 having additional 6 amino acids of Trp-Ile-Ser-Thr-Lys-Leu at the C-terminal of the amino acid sequence;
- e) a polynucleotide sequence having 80% or more sequence identiyy with the polynucleotide sequence coding for an amino acid sequence of SEQ ID NO: 1, or
- f) a polynucleotide sequence that hybridizes with a polynucleotide sequence coding for an amino acid sequence represented by SEQ ID NO: 1 to form a hybrid under high ion concentrations of 450 to 900mM sodium chloride and 45 to 90mM sodium citrate at 65°C and the hybrid formed is maintained as a hybrid after being kept at 65°C for 30 minutes under a low

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ion concentration of 15 to 300mM sodium chloride and 1.5 to 30mM sodium citrate and 0.1 to 1.0 wt% of SDS, the amino acid sequence being an amino acid sequence of an enzyme capable of preferentially producing (S)-4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanoate.

Claim 2 (original): A DNA construct comprising a promoter in operative linkage with the polynucleotide sequence as defined in Claim 1.

Claim 3 (original): A recombinant vector containing the polynucleotide sequence as defined in Claim 1 or 2.

Claim 4 (previously presented): A transformant having the DNA construct as defined in Claim 2.

Claim 5 (original): A transformant according to Claim 4, wherein the transformant is a microorganism.

Claim 6 (original): A transformant according to Claim 5, wherein the microorganism is *E. coli*.

Claim 7 (original): A process for producing a transformant, which comprises the step of introducing the recombinant vector as defined in Claim 3 into a host cell.

Claim 8 (previously presented): A transformant having the polynucleotide as defined in Claim 1.

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Claim 9 (previously presented): A recombinant vector containing
A) a polynucleotide construct as defined in Claim 1, and
B) a polynucleotide coding for an enzyme capable of converting oxidized β -nicotinamide-adenine dinucleotide phosphate into a reduced form, wherein the enzyme is glucose dehydrogenase.

Claim 10 (canceled).

Claim 11 (previously presented): A transformant having the vector according to Claim 9.

Claim 12 (original): A transformant according to Claim 11, wherein the host is a microorganism.

Claim 13 (original): A transformant according to Claim 12, wherein the microorganism is *E. coli*.

Claim 14 (previously presented): A transformant having
A) the polynucleotide as defined in Claim 1, and
B) a polynucleotide coding for an enzyme capable of converting oxidized β -nicotinamide-adenine dinucleotide phosphate into a reduced form, wherein the enzyme is glucose dehydrogenase.

Claim 15 (original): A protein having:
i) an amino acid sequence of SEQ ID NO: 1;
ii) an amino acid sequence encoded by a polynucleotide sequence that hybridizes under stringent conditions with a polynucleotide sequence of SEQ ID NO: 2 coding for an amino acid

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sequence of a protein capable of preferentially producing (S)-4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanoate; or

iii) an amino acid sequence of SEQ ID NO: 1, wherein one or more amino acids are deleted, replaced or added, said amino acid sequence being an amino acid sequence of a protein capable of preferentially producing (S)-4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanoate.

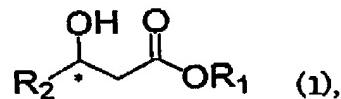
Claim 16 (previously presented): A process for producing (S)-4-halo-3-hydroxybutanoate, which comprises reacting 4-halo-3-oxobutanoic acid ester with the protein as defined in Claim 15, a transformant, which produces said protein or a treated product thereof.

Claim 17 (original): A process according to Claim 16, which comprises allowing the coexistence of an enzyme capable of converting the oxidized β -nicotinamide-adenine dinucleotide phosphate into a reduced form.

Claim 18 (original): A process according to Claim 17, wherein the enzyme capable of converting an oxidized β -nicotinamide-adenine dinucleotide phosphate into a reduced form is a glucose dehydrogenase.

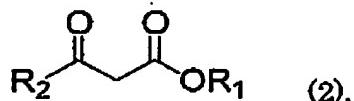
Claim 19 (previously presented): A process according to Claim 17, wherein the 4-halo-3-oxobutanoic acid ester is contacted with the transformant as defined in any one of Claims 11 to 14 or a treated product thereof.

Claim 20 (previously presented): A process according to Claim 16, 17, 18 or 19, wherein the 4-halo-3-oxobutanoic acid ester is represented by a formula (1):



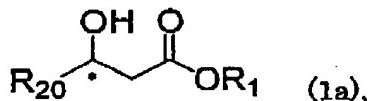
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wherein R₁ represents an alkyl group, and R₂ represents a methyl group which is substituted with a halogen atom, which process comprises reacting 4-halo-3-oxobutanoic acid ester of formula (2):

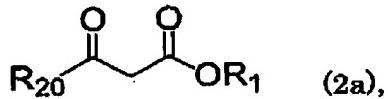


wherein R₁ and R₂ represent the same as defined above.

Claim 21 (previously pending): A process for producing an optically active 3-hydroxybutanoic acid ester of formula (1a):



wherein R₁ represents an alkyl group, and R₂₀ represents a methyl group which may be substituted with a halogen atom, which process comprises reacting 3-oxobutanoic acid ester of formula (2a):



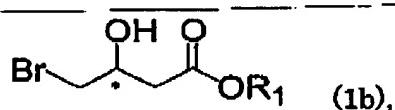
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wherein R₁ and R₂₀ represent the same as defined above, with whole cells of a microorganism or a treated product thereof, which microorganism belongs to *Penicillium citrinum*, *Cryptococcus humicola*s, or *Bacillus alvei* and is capable of asymmetrically reducing the oxo group at 3-position of the compound of formula (2a) to corresponding 3-hydroxy group.

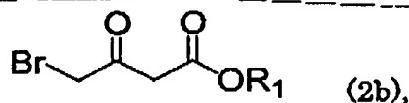
Claim 22 (original): A process according to claim 21, wherein R₂ represents a halomethyl group.

Claim 23 (previously presented): A process according to claim 21 or 22, wherein the microorganism is a strain selected from the group of *Penicillium citrinum* (IFO4631), *Cryptococcus humicola*s (IFO1527), and *Bacillus alvei* (IFO3343t).

Claim 24 (original): A process for producing an optically active 4-bromo-3-hydroxybutanoate of formula (1b):



wherein R₁ represents a (C2-C8) alkyl group, which process comprises reacting 4-bromo-3-oxobutanoate of formula (2b):



wherein R₁ represents the same as defined above, with an enzyme having:

- iv) an amino acid sequence of SEQ ID NO: 34;

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v) an amino acid sequence encoded by a polynucleotide sequence that hybridizes, under stringent conditions, with a polynucleotide sequence of SEQ ID NO: 34, wherein said amino acid sequence is an amino acid sequence of a protein capable of preferentially producing optically active 4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanoate; and

vi) an amino acid sequence of SEQ ID NO: 3, wherein one or more amino acids are deleted, replaced or added, said amino acid sequence being an amino acid sequence of a protein capable of preferentially producing optically active 4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxbutanoate.

Claim 25 (original): A process for producing 4-cyano-3-hydroxybutanoic acid, which comprises reacting 4-bromo-3-hydroxybutanoic acid ester with a metal cyanide in the presence of an alkaline earth metal hydroxide and an alkaline earth metal halogenide.

Claim 26 (previously presented): A process according to Claim 25, which further comprises the step of reacting the 4-cyano-3-hydroxybutanoic acid with dialkyl sulfate to produce 4-cyano-3-hydroxybutanoic acid alkyl ester.

Claim 27 (previously presented): A process according to Claim 25 or 26, wherein the alkaline earth metal hydroxide is calcium hydroxide, and the alkaline earth metal halogenide is calcium chloride.

Claim 28 (previously presented): A process according to Claim 25, wherein the 4-bromo-3-hydroxybutanoic acid ester is (C1-C8) alkyl 4-bromo-3-hydroxybutanoate, and the dialkyl sulfate is dimethyl or diethyl sulfate.

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Claim 29 (previously presented): A process according to Claim 25 or 26, wherein the 4-bromo-3-hydroxybutanoic acid and 4-cyano-3-hydroxybutanoic acid are optically active compounds.

Claim 30 (currently amended): A process according to Claim 25 or 26, wherein the 4-bromo-3-hydroxybutanoic 4-bromo-3-hydroxybutanoic acid is (S)-4-bromo-3-hydroxybutanoic acid ester and 4-cyano-3-hydroxybutanoic acid is (R)-4-cyano-3-hydroxybutanoic acid.

Claim 31 (currently amended): A process for producing (R)-4-cyano-3-hydroxybutanoic acid, which comprises
producing (S)-4-bromo-3-hydroxybutanoic acid ester by asymmetrically reducing the 4-bromo-3-oxobutanoic acid ester, and
reacting (S)-4-bromo-3-hydroxybutanoic acid ester with a metal cyanide in the presence of an alkaline earth metal hydroxide and an alkaline earth metal halogenide.

Claim 32 (previously presented): A process according to Claim 31, wherein the asymmetrical reduction is conducted by a microorganism or treated product thereof capable of asymmetrically reducing the 4-bromo-3-oxobutanoic acid ester to (S)-4-bromo-3-hydroxybutanoic acid ester.

Claim 33 (previously presented): A process according to Claim 32, wherein the microorganism is a microorganism belong to *Penicillium citrinum*.

Claim 34 (currently amended): A process according to Claim 31, 32 or 33, wherein (S)-4-bromo-3-hydroxybutanoic 4-bromo-3-hydroxybutanoic acid ester and 4-bromo-3-oxobutanoic acid ester are (C1-C8) alkyl ester.

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Claim 35 (previously presented): A process according to Claim 33, wherein the microorganism is a strain *Penicillium citrinum* (IFO 4631).

Claim 36 (previously presented): A process according to any one of Claim 31 to 35, wherein the alkaline earth metal hydroxide is calcium hydroxide and the alkaline earth halogenide is calcium chloride.

Claim 37 (currently amended): A process according to Claim 31, which further comprises the step of reacting (R)-4-cyano-3-hydroxybutanoic acid with dialkyl sulfate to produce ~~(R)-4-cyano-3-hydroxybutanoic acid~~ (R)-4-cyano-3-hydroxybutanoic acid alkyl ester.

Claim 38 (previously presented): A process according to Claim 32, wherein the alkyl group of the dialkyl sulfate is a methyl or ethyl group.

Claim 39 (previously presented): A transformant having the vector as defined in Claim 3.

Claim 40 (previously presented): A recombinant vector according to Claim 9, wherein the enzyme in B) is glucose dehydrogenase derived from *Bacillus megaterium*.

Claim 41 (previously presented): A transformant according to Claim 14, wherein the enzyme is glucose dehydrogenase derived from *Bacillus megaterium*.